

We Claim:

1. A system for optical detection of emitted radiation from microlocations on an object to be examined, comprising:

a light source providing excitation radiation characterized in that the diameter of the excitation radiation when illuminating the microlocation is of the same diameter or less than the diameter of the microlocation to be examined,

a confocal scanning system adapted to receive the excitation radiation and direct it to the microlocation and to provide reflected radiation and emitted radiation from the microlocation,

a first detector adapted to receive the reflected radiation and a position detection system, the first detector having an output connected to a position detection system,

a second detector operatively positioned to receive the emitted radiation from the microlocation, the detector characterized in that the diameter examined by the detector is less than or equal to the diameter of the excitation radiation, and

a control system coupled to the position detection system which causes the confocal scanning system to direct excitation radiation to a specific microlocation.

2. The system of claim 1 wherein the excitation source is a laser.

3. The system of claim 2 wherein the laser is a single laser source.

4. The system of claim 1 wherein the scanning system is an x-y scanning system.

5. The system of claim 4 wherein the x-y scanning system includes a mirror adapted to reflect the excitation radiation, the reflected radiation and the emitted radiation.

6. The system of claim 1 wherein the second detector further includes an aperture.
7. The system of claim 6 wherein the aperture comprises a pinhole aperture.
8. The system of claim 7 wherein the pinhole aperture corresponds to a microlocation with a diameter in the range from substantially 20 microns to 80 microns.
9. The system of claim 8 wherein the pinhole aperture corresponds to a microlocation with a diameter of substantially 50 microns.
10. The system of claim 1 wherein the second detector includes a photomultiplier tube.
11. The system of claim 1 further including a focusing motor.
12. The system of claim 1 further including a rejection filter disposed between the confocal scanning system and the second detector.
13. The system of claim 12 wherein the rejection filter rejects excitation radiation to a factor of 10^7 .
14. The system of claim 12 wherein the rejection filter rejects excitation radiation to a factor of 10^{10} .
15. The system of claim 1 further including a data acquisition system.
16. The system of claim 1 further including a display.

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17. The system of claim 1 further including a laser power monitor positioned to receive excitation radiation and output an indication of laser power.

18. The system of claim 17 wherein the output of the laser power monitor is connected to the control system.

19. A confocal microscopy system including an optical detection platform comprising:

a restricted excitation source characterized in that the diameter of the excitation radiation incident upon the object to be examined is less than the lateral dimension of the object to be examined and at least substantially five times greater than the diffraction limited spot size of the excitation source, and

a detector characterized in that it has a restricted field of view in diameter which is no larger than the diameter of the excitation radiation incident upon the object to be examined.

20. The confocal microscopy system of claim 19 wherein the excitation source is a laser.

21. The confocal microscopy system of claim 20 wherein the diameter of the laser at the surface of the object to be examined is less than substantially 80 microns.

22. The confocal microscopy system of claim 20 wherein the diameter of the laser at the surface of the object to be examined is greater than substantially ten times the diffraction limited spot size of the excitation source.

23. The confocal microscopy system of claim 20 wherein the diameter of the laser at the surface of the object to be examined is greater than substantially 20 microns.

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24. The confocal microscopy system of claim 20 wherein the diameter of the laser at the surface of the object to be examined is greater than substantially 40 microns.

25. The confocal microscopy system of claim 20 wherein the diameter of the laser at the surface of the object to be examined is approximately 50 microns.

26. The confocal microscopy system of claim 20 wherein the diameter of the laser at the surface of the object to be examined is in the range from 100 microns to 50 microns.

27. A method for optically examining a microlocation on an object comprising the steps of:

illuminating at least a portion of the object by scanning light from a source through a scanning confocal microscope onto the object, and detecting light reflected from the object through the scanning confocal microscope,

determining the position of the microlocation by analyzing the detected reflected light,

illuminating a microlocation with light from the source through the scanning confocal microscope utilizing the determined position of the microlocation, and

detecting emitted radiation from the microlocation.

28. The method of claim 27 for optically examining a microlocation wherein the step of determining the position of the microlocation includes use of information regarding the microlocation patterns.

29. The method of claim 27 for optically examining a microlocation wherein the step of illuminating the microlocation illuminates no more than a single microlocation.

30. The method of claim 27 for optically examining a microlocation wherein the step of detecting emitted radiation is subject to a field of view restricted to a microlocation.

Sub 31. A method for examining an object having multiple microlocations separated by interstitial areas comprising the steps of:

illuminating multiple points on the object to be examined,
detecting reflected radiation from the object to be examined,
comparing the information constituting reflected radiation with information regarding the structure of the object to be examined, whereby the position of the object is determined, and
illuminating one microlocation through a confocal microscope based upon the position information.

32. The method of claim 31 for alignment of an object having multiple microlocations wherein the multiple points of detection are gathered by scanning the radiation over at least two microlocations and one interstitial area.

33. A method for determining fluorescence intensity from multiple microlocations disposed on the surface of a biological diagnostic system comprising the steps of:

scanning the surface of the diagnostic system which includes the microlocations with a laser source directed through a scanning confocal optical system,

detecting light reflected from the microlocations,
determining the position of the microlocations by imaging the reflected light,
illuminating one microlocation through the confocal optical system based upon the determined position, wherein the illumination does not extend substantially beyond the microlocation, and

detecting from the microlocation via the confocal microscope, where the detector masks emissions from the object in regions other than the one

~~microlocation.~~

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